1 A case of transplacental transmission of *Theileria equi* in a foal in Trinidad

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17 Abstract

18 Equine piroplasmosis due to *T. equi* and *B. caballi* is endemic in Trinidad. A case
19 of equine piroplasmosis due to *T. equi* was diagnosed in a Thoroughbred foal at 10 hrs
20 post-partum. A high parasitaemia (63 %) of piroplasms was observed in a Wright-
21 Giemsa® stained thin blood smear from the foal. In addition, the 18S rRNA gene for
22 *Babesia/Theileria* was amplified from DNA extracted from the blood of the foal and the
23 mare. Amplified products were subjected to a reverse line blot hybridization assay
24 (RLB), which confirmed the presence of *T. equi* DNA in the foal. The mare was negative
25 by RLB but was positive for *T. equi* using a nested PCR and sequence analysis.

26 In areas where equine piroplasmosis is endemic, severe jaundice in a post-partum
27 foal may be easily misdiagnosed as neonatal isoerythrolysis. Foals with post-partum
28 jaundice should be screened for equine piroplasmosis, which may be confirmed using
29 molecular methods if available.

30 Key words: Neonatal foals, equine piroplasmosis, reverse line blot, Trinidad

Introduction

Equine piroplasmosis is a tick-transmitted haemoparasitic disease of equids caused by the protozoa *Theileria equi* or *Babesia caballi*. Ixodid tick vectors of the genera *Rhipicephalus*, *Hyalomma* and *Dermacento*r are known to transmit the disease (de Waal, 1992). Both parasites are distributed world wide, however several countries restrict the importation of horses that are serologically positive for the disease (Magnarelli et al., 2000) . The clinical signs of equine piroplasmosis are often variable and non-specific. Acute infection results in fever (40°C), depression, reduced appetite, pallor, icterus, dyspnoea, petechiation, sweating, colic, eyelid and distal limb oedema and incoordination (Zobba et al., 2008). Massive destruction of erythrocytes results in haemoglobinuria. Chronic infection tends to result in variable clinical signs (de Waal, 1992).

Equine piroplasmosis can also be transmitted iatrogenically via the common use of blood contaminated syringes and needles and this route is thought to be responsible for the 2008 outbreak of the disease in several horses in Florida (Florida Department of Agriculture and Consumer Services Division of Animal Husbandry, 2009). Transplacental transmission of *T. equi* has been reported and although suspected, evidence of *B. caballi* infection via vertical transmission *in utero* is lacking (Allsopp et al., 2007). Infection of foals by *T. equi in utero* can result in abortions, full term still births or the birth of live foals with neonatal piroplasmosis (Allsopp et al., 2007; Phipps and Otter, 2004). Equine piroplasmosis due to *B. caballi* and *T. equi* has been reported in adult horses in Trinidad using molecular (Rampersad et al., 2003) and serological (Asgarali et al., 2007) techniques. This is the first report of a case of transplacental transmission of *T. equi* in Trinidad.

Case description:

Diplomacy, a 12-year old thoroughbred mare had a normal pregnancy and parturition. The foal was born weak and severely icteric (Figure 1) with blood tinged urine. Blood from the foal was collected into an EDTA tube for routine haematological analysis at 10 hrs postpartum. A thin blood smear was stained with Wright-Giemsa ® and examined under light microscopy. Piroplasms in red blood cells (RBC) were evident (Figure 2). A stress leukon of lymphopaenia with thrombocytopaenia was evident (Table 1). The foal died 24 hrs after birth. Microscopic examination of a Wright-Giemsa ® stained peripheral blood smear from the mare was negative for piroplasms.

Materials and methods

Reverse line blot (RLB)

DNA was extracted from 100 µL of EDTA blood from the foal and the mare using the DNeasy blood and tissue kit (Qiagen Sciences Maryland, USA) according to the manufactures instructions. The 18S rRNA gene for *Babesia /Theileria* was amplified using primers F2 and R2 as described previously (Gubbels et al., 1999). DNA of *B. canis rossi* was used as a positive control and PCR grade water (Sigma St. Louis MO, USA) was used as a negative control. The amplified products were then subjected to the reverse line blot (RLB) hybridization process (Gubbels et al., 1999).The oligonucleotide probes that were attached to the membrane for the RLB are listed in Table 2.(Matjila et al., 2005; Nagore et al., 2004)

Nested PCR

DNA extracted from the blood of the mare was then shipped to the University of Georgia , USA and subjected to a nested PCR for the *Babesia* or *Theileria* 18S rRNA

77 gene using primary primers 5.1 and B (Medlin et al., 1989; Yabsley et al., 2005) followed
78 by a secondary reaction using primers F and R as described (Gubbels et al., 1999). To
79 prevent and detect contamination, DNA extraction, primary and secondary amplification,
80 and product analysis were done in separate dedicated areas. Two negative water controls
81 were included in each set of DNA extractions and 1 water control was included in each
82 set of primary and secondary PCR reactions. A blood sample from a white-tailed deer
83 (*Odocoileus virginianus*) naturally infected with *Theileria cervi* was used as a PCR-
84 positive control. To confirm identity, the positive sample was sequenced. The amplicon
85 was purified with a commercial gel-purification kit (QIAGEN, Valencia, CA) and
86 submitted to Clemson University Genomics Institute (Clemson, SC) for bi-directional
87 sequencing using the F and R primers.
88 Results
89 A high parasitaemia (63%) was noted in the thin blood smear from the foal. There
90 were on average 1-3 organisms per infected RBC. The RBC and haematocrit (HCT)
91 levels were on the low end of the reference interval(Weiss and Wardrop, 2010). Based on
92 the results of the RLB, the foal was infected with *T. equi* only (Figure 3). The mare tested
93 negative by RLB but an amplicon of ~ 550 bp was obtained by nested PCR analysis. The
94 nucleotide sequence (504 bp) obtained from the mare was 99.8% similar to *T. equi*
95 sequences from equines from Spain (AY 150062) and South Africa (EU 642508,
96 Z15105).
97 Discussion
98 Clinical signs of icterus and haemoglobinuria as well as reductions in the number
99 of red blood cells and platelets, reduction in haemoglobin concentration and leukopaenia

100 are common in equine babesiosis (de Waal, 1992). Hyperbilirubinaemia is due to
101 haemolytic anaemia. Haemolysis may be a result of mechanical damage to erythrocytes
102 by trophozoite intra-erythrocytic binary fission and toxic damage by haemolytic factors
103 produced by the parasite. The foal was anaemic, however the degree of true anaemia may
104 not be reflected on the foal's haemogramme because of the sharp increase in RBC
105 parameters at birth. During the first 12 hrs, transfusion of placental blood to the foal from
106 the mare increases the foals RBC parameters which then decline sharply over 12-24 hrs
107 (Weiss and Wardrop, 2010). Mild anisocytosis is also commonly observed.(Weiss and
108 Wardrop, 2010).

109 Our case has demonstrated that carrier mares can transmit *T. equi* to their foals
110 and such foals born at term can have an overwhelming parasitaemia. If icterus is not
111 observed immediately after birth, these cases of neonatal piroplasmosis could be
112 mistaken for neonatal isoerythrolysis.

113 Allsopp *et al* (2007) suggested that neonatal equine piroplasmosis may be
114 acquired during days 40 – 150 of gestation which corresponds to the histotrophic when
115 the embryo is exposed to a mixture of uterine gland secretions, desquamated epithelial
116 cells and maternal erythrocytes (histotroph). A study which used medically induced
117 abortions in *T. equi* serologically positive mares, detected *T. equi* DNA in all aborted
118 foetuses. In that study, the earliest abortion was at day130 of gestation (Allsopp et al.,
119 2007). However, microscopic examination of peripheral blood smears was not performed
120 in that study, hence the level of parasitaemia in the aborted foals and mares was
121 unknown. A report of transplacental transmission of *T. equi* has been described in
122 asymptomatic horses with no known natural exposure to the tick vector, thus indicating

123 that such carrier animals may act as reservoirs to establish equine piroplasmosis in areas
124 where competent tick vectors may be present (Phipps and Otter, 2004). The severely
125 icteric foal in our study had a 63% parasitaemia of *T. equi*, however, no piroplasms were
126 observed on light microscopy of Wright-Giemsa® stained peripheral blood smears of the
127 mare. The RLB was also not sensitive enough to detect *T. equi* DNA in the blood of the
128 mare but *T. equi* was detected using a nested PCR. The negative RLB result in the mare
129 in our study may be due to a very low level of parasitaemia which was below the limit of
130 detection of the RLB. The detection limit for the RLB for bovine piroplasms was
131 estimated by Gubbels *et al* to be 10^{-6} % which corresponded to 3 parasites per µl of blood
132 (Gubbels et al., 1999).
133 The economic importance of transplacental transmission of equine piroplasmosis
134 on the thoroughbred industry in Trinidad needs further investigation as many cases may
135 be misdiagnosed. We therefore recommend that all icteric foals born in endemic areas
136 should be screened for neonatal piroplasmosis. This is the first reported case of neonatal
137 piroplasmosis in Trinidad.
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Figure 1 Icteric sclera and conjunctiva of neonatal foal at 10 h post- partum

Figure 2 Red blood cells (RBC) of foal infected with *T. equi* piroplasms. There were 126 infected RBCs per high power field (HPF). There are approximately 200 RBC per HPF (100x oil objective).

Figure 3 RLB hyperfilm result for foal diplomacy (b) (1, *Babesia/Theileria* genera probe; 2, *Babesia canis rossi*; 3, *Babesia caballi*; 4, *Theileria equi*). a, b, c (equine samples), d positive control DNA containing *B. canis rossi*, e (negative control)

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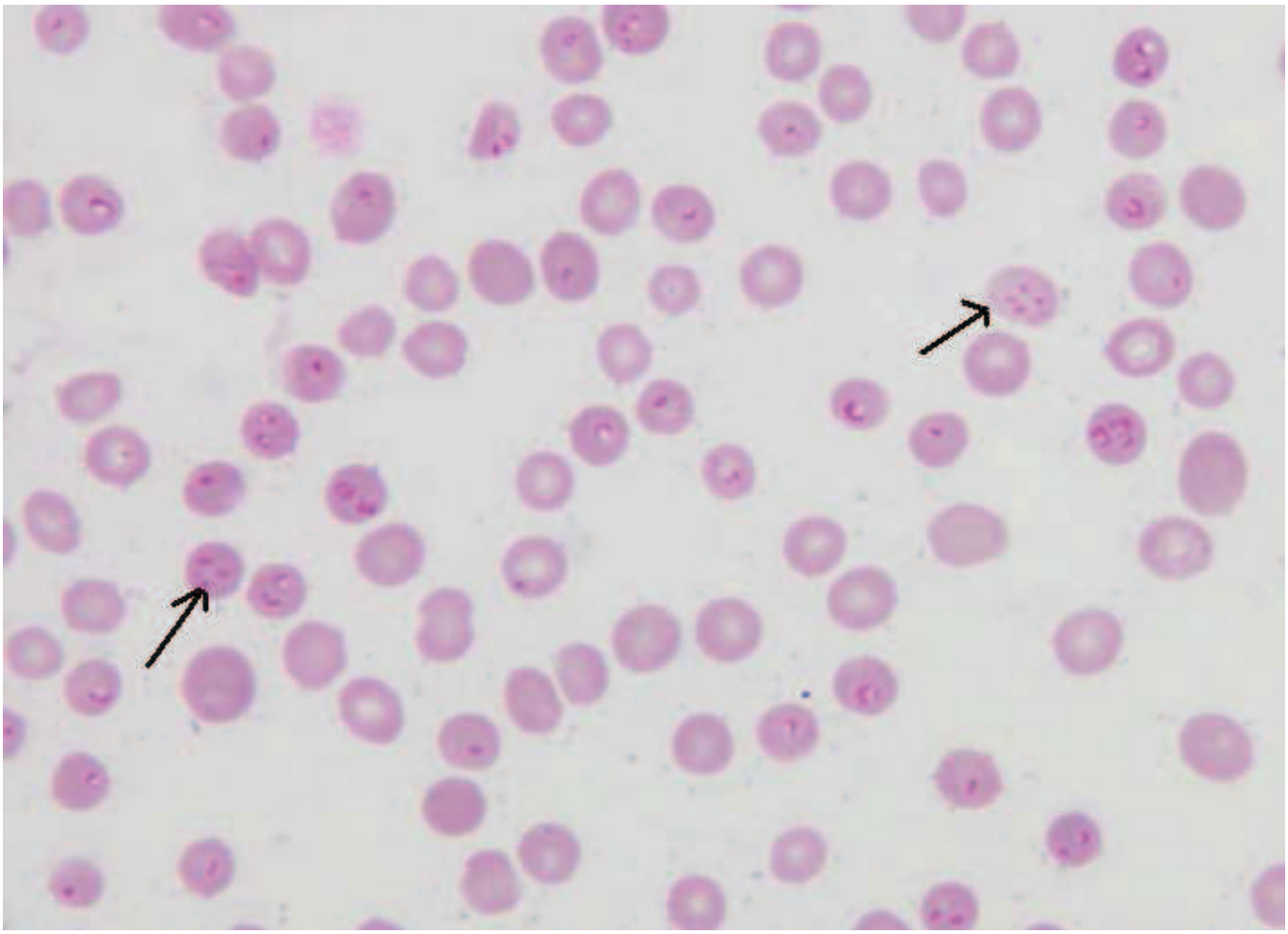
Table 1 The foal's haematological parameters at 10 hours post- partum

Variable – Reference interval for Thoroughbred foals at 24 hrs[1]	
RBC (x 10^{12} / L) – (9.1 – 11.9)	7.4
Hgb (g/L) – (129.0-155.0)	107.0
HCT(L/L) – (0.38 - 0.46)	0.34
MCV (fl) – (36.0 – 44.0)	46.0
MCH(pg) - (13.0 – 15.0)	14.4
Anisocytosis	2+
MCHC (g/L) – (320.0 – 360.0)	312.0
WBC (x 10^9 / L) – (6.0 – 12.0)	5.0
Neutrophils (%) – (58.0-79.0) (x 10^9 / L) – (4.0-9.5)	74.0 3.7
Bands (%) – (0 – 3.0) (x 10^9 / L) – (0 - 0.1)	4.0 0.2
Metamyelocytes (%) – (0) (x 10^9 / L) - (0)	1.0 0.05
Lymphocytes (%) – (15.0 – 35.0) (x 10^9 / L) – (1.3-3.1)	10.0 0.5
Monocytes (%) – (1.0-7.0) (x 10^9 / L) – (0 -0.6)	9.0 0.45
Eosinophils (%)- (1.0-10.0) (x 10^9 / L)- (0 - 0.8)	0
Platelets (x 10^9 / L) – (100.0 – 600.0)	58.0
Protein (g/L) – (53.0 – 71.0)	68.0
Fibrinogen (g/L) – (2.1- 3.3)	5.0

RBC = red blood cell, Hgb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, WBC = white blood cell. [1] Adapted from Schalm's Veterinary Hematology, Sixth Edition (ed Weiss and Wardrop, 2010)

Figure 1 Icteric sclera and conjunctiva of neonatal foal at 10 hrs post-partum



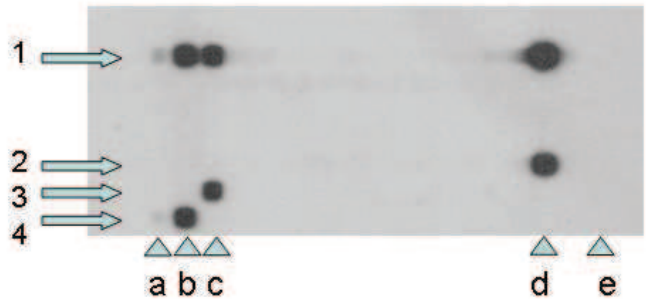

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Table 2. 5'-3' sequence of oligonucleotide probes used in this study[2]

Oligonucleotide probe	5'-3' sequence of oligonucleotide probe	Reference
Babesia/*Theileria genera*	TAATGGTTAATAGGARCRGTTG	(Gubbels et al., 1999)
Babesia caballi	CGGGTTATTGACTTCGCTTTTTCTT	(Nagore et al., 2004)
Babesia canis rossi	CGGTTTGTTGCCTTTGTG	(Matjila et al., 2005)
Theileria equi	TCTGCTGTTTCGTTGACTG	GenBank Accession no. Z15105, AY150062

[2] R = A or G